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SUMMARY

While chemoautotrophic endosymbioses of hydrothermal vents and other reducing environments have been well studied, little attention has been paid to the magnitude of the metabolic demands placed upon the host by symbiont metabolism, and the adaptations necessary to meet such demands. Here we make the first attempt at such an evaluation, and show that moderate to high rates of chemoautotrophic or methanotrophic metabolism impose oxygen uptake and proton equivalent elimination demands upon the hosts that are much higher than is typical for the non-symbiotic annelid, bivalve, and gastropod lineages to which they are related. The properties of the hosts are described and compared to determine which properties are associated with and predictive of the highest rates. We suggest that the high oxygen demand of these symbionts is perhaps the most limiting flux for these symbioses. Among the consequences of such demands has been the widespread presence of circulating and/or tissue hemoglobins in these symbioses that are necessary to support high metabolic rates in thioautotrophic endosymbioses. We also compare photoautotrophic with chemo- and methanotrophic endosymbioses to evaluate the differences and similarities in physiologies. These analyses suggest that the high demand for oxygen by chemo- and methanotrophic symbionts is likely a major factor precluding their endosymbiosis with cnidarians.

Key words: chemoautotrophy, photoautotrophy, symbiosis, Cnidaria, Anthozoa, *Riftia*, oxygen consumption, hemoglobin, sulfide.

Introduction

The deep-sea hydrothermal vent communities were discovered in 1977 and immediately recognized as radically different ecosystems in the deep sea (Corliss and Ballard, 1977; Corliss et al., 1979). Unlike the rest of the deep sea, these communities exhibited extremely high biomasses, aggregated in small areas, whose dominant species were very large and taxonomically novel. By early 1980, the “secret” of these dominant species was found to be endosymbiotic relationships with chemoautotrophic microorganisms whose primary production was fueled by the oxidation of hydrogen sulfide (Cavanaugh, 1985; Cavanaugh et al., 1981; Felbeck, 1981). Subsequent exploration revealed that these symbioses are found in other chemically reducing habitats and in a variety of taxa (for review see Dubilier et al., 2008; Stewart et al., 2005). Although most of the symbionts are sulfur-oxidizers, a number of methanotrophic symbionts have also been found.

From early on in vent research it was apparent that the giant tubeworm, *Riftia pachyptila*, had unusually high growth rates (Lutz et al., 1994). As they lack a mouth or gut as an adult, *Riftia* (a monospecific genus) must have high rates of carbon fixation to support their growth. The physiological functioning of hydrothermal vent species, especially *Riftia pachyptila*, was studied intensively in following years and major aspects of its physiology and biochemistry were discerned (Arp et al., 1985; Childress and Fisher, 1992). Studies showed that hemoglobins play a key role in this physiological functioning, binding both sulfide and oxygen to separate sites, preventing spontaneous oxidation and allowing their transport to the symbionts (Arp and Childress, 1983; Arp et al., 1987; Childress et al., 1991a). Many years of effort were required, however, to successfully measure net fluxes of the major metabolites in these symbioses, as high pressure was necessary to sustain physiological function (Childress et al., 1991; Girguis and Childress, 2006).

Studies of chemoautotrophic symbioses have revealed a range of metabolic rates that generally correspond to the availability of reducing substrates in the animals’ environments and the observed growth rates of the animals. These publications have primarily emphasized the rates of net uptake of inorganic carbon and sulfide. Notably, the intent of this review is to consider the relatively unexamined quantitative demand for the primary oxidant, oxygen, in these symbioses in the context of their physiological functioning (while studies have shown that nitrate is clearly important as a N source for the symbionts, it does not appear to be an important oxidant). It is apparent that chemoautotrophy is very demanding of oxygen, and a previous study suggests that up to 80% of oxygen uptake is driven by symbiont metabolism (Girguis and Childress, 2006)). Thus, to sustain high rates of sulfide or methane oxidation, and in turn net carbon incorporation, these hosts must be able to sustain high rates of oxygen uptake by the host and high rates of oxygen transport to the symbionts. Here we propose that the capacity for rapid and continuous uptake of oxygen to support symbiont metabolism is a crucial adaptation for chemoautotrophic and methanotrophic endosymbioses, which severely restricts the ability of some invertebrate taxa to evolve such endosymbioses. Moreover, the ability to cope with and eliminate proton equivalents resulting from chemoautotrophic function is also essential. We also propose that this provides a reasonable explanation for the absence of chemoautotrophic symbioses in the Cnidaria, the phylum with the greatest diversity of photoautotrophic endosymbioses.

Comparing physiological and morphological attributes of chemoautotrophic and photoautotrophic endosymbioses

Although chemoautotrophic and methanotrophic symbioses have been described in many metazoan taxa (Dubilier et al., 2008; Stewart et al., 2005), only the siboglinid annelids and bivalve and gastropod molluscs have sulfur or methane oxidizing symbionts within host cells (bacteriocytes). In the siboglinids, the symbiont bacteriocytes are located in a specialized tissue within the body called the trophosome (Jones et al 1981). This organ is far removed from the gill, or plume. In contrast, in molluscs - wherein six different families have independently evolved endosymbioses with chemoautotrophs- the bacteria are contained in bacteriocytes located at the gill surfaces (Stewart et al., 2005).

Similarly, among photoautotrophic symbioses (which are widely distributed among metazoan taxa), only the Cnidaria and gastropod mollusks have intracellular symbionts (Smith and Douglas, 1987). Of these, the Cnidaria clearly have the greatest proliferation of symbiotic species as well as dominance in some ecosystems. In the cnidarians, the symbionts are contained in cells of the endoderm. In the opisthobranch molluscs, which lack gills, the chloroplasts are contained in cells lining the digestive tract that are very close to the surface.

All these intracellular autotrophic symbioses share the requirement that substrates and endproducts of symbiont metabolism must pass through the animal's tissues. This presents an opportunity for the hosts to facilitate the functioning of the endosymbionts (which is discussed in greater detail below). For photoautotrophic symbioses, sunlight is necessary for phosynthesis, and as such the endosymbionts are located near the surface of the animals where light can readily penetrate the tissues. The chemoautotrophic symbioses, on the other hand, must supply a reduced sulfur compound (sulfide or thiosulfate) as well as oxygen to support chemosynthesis. While sunlight is not required for chemosynthesis, there are still advantages to locating the symbionts near the host's surface, and this is evident in the body plan of endosymbiotic molluscs. The siboglinid trophosome is located deep within the worm (also discussed in detail below). With respect to eliminating waste products from symbiont metabolism, photoautotrophic symbioses must dispose of excess oxygen produced during photosynthesis. Chemoautotrophic hosts must eliminate the endproducts of sulfur oxidation, mainly sulfate and hydrogen ions (Goffredi et al., 2000; Girguis et al., 2002). Among photoautotrophs, symbiont photosynthesis results in high internal oxygen partial pressures in high light regimes, which drives diffusion of oxygen. For chemoautotrophic symbioses, sulfate and proton equivalents are actively "pumped" out against the concentration gradient. Notably, both types of symbioses require defenses against reactive oxygen species produced during photosynthesis (Shick and Dykens, 1985) or sulfur oxidation (Blum and Fridovich, 1984; Tapley and Shick, 1991).

In both types of symbioses, nitrogen is often taken up by the symbionts in the form of ammonium ions, either from the environment or from the catabolism of food captured by the host (Lee and Childress, 1994; Miller and Yellowlees, 1989; Yellowlees et al., 2008). Some photoautotrophic symbioses are also able to take up nitrate from the very low concentrations found in their environments (Furla et al., 2005). Many photoautotrophic symbioses occupy nutrient poor habitats, and depend on obtaining ammonium from heterotrophic feeding and recycling within the symbiosis as well as from the environment (Falkowski et al., 1993; Yellowlees et al., 2008). In the chemoautotrophic and methanotrophic symbioses, most examined are able to readily use nitrate, which is much more available in deep ocean waters (Lee and Childress, 1994; Girguis et al, 2000). The siboglinids appear to use only nitrate because they maintain very high internal ammonium concentrations throughout their bodies due to their uptake of nitrate and its reduction to ammonium by the symbionts (De Cian et al., 2000; Girguis et al., 2000). In addition, nitrogen limitation is considered a possible mechanism for the photoautotrophic host's control of symbiont density (Falkowski et al., 1993). This is unlikely to be the case for most chemoautotrophic symbioses because of the ready availability of inorganic nitrogen in their environments as well as high internal ammonium concentrations in siboglinids.

With respect to carbon acquisition, photoautotrophic and chemoautotrophic symbioses typically host symbionts that fix inorganic carbon. For both photoautotrophic and chemoautotrophic symbioses, inorganic carbon is derived from the ambient seawater, which typically contains ca. 2 mmol l^{-1}, as well as from the animal respiration. At vents and seeps, however, chemoautotrophic symbioses also acquire their inorganic carbon from a mixture of bottom water and porewaters or vent fluids. In these mixed, diffuse fluids inorganic carbon can reach greater than 6 mmol l^{-1} and pH values of 6 to 6.5 around *Riftia* (Childress et al., 1993) with low pH being typical of hard bottom deep-sea vent environments (Tunnicliffe et al., 2009). At vents and seeps, elevated inorganic carbon and lower environmental pH results in increased pCO_2 (Childress et al., 1993b), which can greatly increase the ability of the chemoautotrophic symbioses to take up inorganic carbon. The elevated external pCO_2 results in high internal pCO_2 which is expected to be much more important as a resource for C fixation than the much smaller amount of respiratory CO_2 produced (in chemoautotrophs, respiratory CO_2 is mostly if not entirely derived from host respiration of symbiont produced carbon, and does not contribute to net productivity). Notably, the uptake of inorganic carbon in both types of associations is facilitated by carbonic anhydrases, which catalyze the rapid interconversion of bicarbonate and carbon dioxide (Goffredi et al., 1999b; Kochevar and Childress, 1996; Yellowlees et al., 2008).

The reduced sulfur compound hydrogen sulfide is extremely toxic to animals as it poisons cytochrome-c-oxidase and arrests aerobic respiration. In general, photoautotrophic symbioses are not exposed to reduced chemicals such as hydrogen sulfide. As such, most are not likely adapted to mitigate exposure to sulfide. In contrast, chemoautotrophic symbioses live in environments characterized by substantial sulfide concentrations. All the inhabitants of these habitats -whether they have symbionts or not- must deal with the problem of sulfide toxicity. In the case of the symbiotic molluscs, they typically oxidize sulfide to thiosulfate to reduce toxicity, and their symbionts can use thiosulfate (which is significantly less toxic) as a reductant. However, only siboglinids have been shown to exclusively transport sulfide to the symbionts, having negligble production of thiosulfate (Childress and Fisher, 1992; Childress et al., 1991a). Hemoglobin is typically very abundant in siboglinids as well as all of the molluscan chemoautotrophic symbioses except the mytilid bivalves (Dando et al., 1985; Doeller et al., 1988; Terwilliger and Terwilliger, 1985; Wittenberg, 1985; Wittenberg and Stein, 1995). These respiratory pigments have been implicated in the supplying of oxygen and sulfide to the endosymbionts in these groups, and will be discussed in detail later. To date, none of the photoautotrophic endosymbioses have been found to contain hemoglobin or other respiratory pigments.

Another very interesting difference between the photoautotrophic and chemoautotrophic symbioses is the means by which the host obtains reduced carbon compounds from the endosymbionts. In the case of the photoautotrophic symbioses, it seems to universally be the case that the endosymbionts “leak” one or a few specific organic compounds under the control of the hosts (Trench, 1993; Venn et al., 2008; Yellowlees et al., 2008). For the chemoautotrophic symbioses, the hosts appear to digest the endosymbionts in all the groups except the bivalves of the family Solemyidae (Bright et al., 2000; Fiala-Medioni et al., 1994). In the case of the methanotrophic mussel, *Bathymodiolus childressi*, this transfer takes days supporting histological evidence for digestion (Fisher and Childress, 1993). In the case of the solemyid *Solemya reidi*, the movement of ^{14}C labeled organic carbon from the gills takes place within minutes precluding digestion (Fisher and Childress, 1986).

Among the chemoautotrophic symbioses, only the siboglinid polychaetes are organized in such a fashion that the endosymbionts are remote from the surface of the animal hosts and therefore metabolites are passed through multiple tissues as well as being transported in the vascular system on their way to the symbionts (Jones, 1981). The closest to this organization in the photoautotrophic symbioses are tridacnid clams that have extracellular symbionts contained in extensions of the digestive tract in the mantle. These tubular extensions are in very close association with the vascular system so that photosynthetically produced oxygen is removed by the circulatory system and gills (Farmer et al., 2001; Mangum and Johansen, 1982). In both cases, such an organization allows the animal hosts to control the supply of metabolites to the endosymbionts as well as effectively remove waste products. It also potentially enables much higher rates of metabolite uptake and transport to and from the endosymbionts as well as animal tissues.

In light of the hosts' dependence on symbiont primary production, comparing differences in carbon fixation rates among these photoautotrophic and chemoautotrophic symbioses is especially revealing. The data presented here for chemoautotrophic symbioses (Table 1) represent net fluxes of metabolites measured using flow-through pressurized aquaria. Heterotrophic metabolism by the host has been subtracted out of the carbon flux data, so these values represent net production (i.e. carbon accumulation, that is growth) in relation to wet body mass. Primary production rates by intact photoautotrophic symbioses have mostly been inferred from the net oxygen production while illuminated, oxygen consumption in the dark, estimates of % translocation of photosynthate and other factors. To our knowledge, all such data have been normalized to something other than live weight, usually protein or chlorophyll (Falkowski et al., 1984; Muscatine and Porter, 1977; Yellowlees et al., 2008). Regardless, the net rates of carbon fixation by chemoautotrophic associations (Table 1) can roughly be compared to those of the photoautotrophs with respect to the rates of heterotrophic CO_2 production. In most cases, the rates of sustained net inorganic carbon uptake in chemoautotrophic symbioses exceeds heterotrophic production by several fold, varying from about 100% of the heterotrophic consumption (i.e. gross uptake is about twice the heterotrophic rate) up to 10 to 14 times higher for the siboglinids and *Alviniconcha*.

Photoautotrophic net carbon fixation estimates are also quite variable, though these it has been suggested that symbiont carbon fixation may not always account for the associations' carbon needs. For photoautotrophic symbioses it appears that the maximum gross primary production as estimated from oxygen production would be less than twice the heterotrophic consumption (Falkowski et al., 1984). For example, using data from McCloskey and Muscatine (1984), it is possible to make an approximate estimate of net inorganic C uptake as % of body C for the coral *Stylophora pistillata*, (McCloskey and Muscatine, 1984). These authors report that specimens of this species had net C fixation rates of 0.698 and 0.168 mg C mg^{-1} algal C day^{-1} respectively for specimens from 3 and 35 m. Using the biomass ratios of 5.1% and 4.0% respectively (as in Falkowski et al. 1984) we can calculate that this species has net inorganic C fixation rates of 3.6 and 0.67% of total body carbon per day respectively. From these data, it appears that cnidarian algal endosymbioses can have C fixation rates relative to body C that are comparable to the less productive chemoautotrophic associations, but well below those of the most effective symbioses, the siboglinids and *Alviniconcha* (ca. 10% of body carbon per day). In both photoautotroph and chemoautotrophic associations, the degree to which heterotrophy supplements symbiont-derived organic matter has been well studied. Notably, all of the cnidarian symbioses and nearly all photoautotrophic symbioses feed heterotrophically. In contrast, all of the chemoautotrophic endosymbioses -except the mytilid bivalves- have severely reduced or no ability to feed on particulate material, emphasizing their greater dependence upon carbon fixed by their endosymbionts. While it is generally accepted that the leaked carbon in the photoautotrophic symbioses is not nutritionally complete (Falkowski et al., 1984), this is not likely the case in those chemoautotrophic symbioses that digest their symbionts and cannot feed.

Comparison of the rates of metabolite exchange among chemoautotrophic endosymbioses.

In comparing the metabolite fluxes of chemoautotrophic symbioses shown in Table 1, we will explore the anatomical organizations and physiological properties that make these fluxes possible. It is also essential to consider the availability of reduced substrate in the species' habitats as some habitats such as hydrothermal vents have very large amounts of sulfide available while others such as reducing sediments are constrained by the low rates of diffusion through sediment. The rates presented in Table 1 are sustained net fluxes, determined in flowing water respirometers, at habitat pressure for vent species, over periods of hours to days. Host metabolism is part of the oxygen uptake rates, but not part of the inorganic carbon uptake rates which are net rates. As mentioned, the animal metabolic demands in highly productive symbioses are much lower than those of the symbionts. For example, at the temperatures shown, the oxygen consumption due to *Riftia* host respiration is about 2 µmol g^{-1} h^{-1} (Childress et al., 1984; Childress and Mickel, 1985) while the remaining 27 µmol g^{-1} h^{-1} represents the oxidation of sulfide by the symbionts (Table 1). Notably, the rates of oxygen consumption by these symbioses when in autotrophic balance (meaning when chemoautotrophic metabolism exceeds heterotrophic metabolism and produces a net uptake of inorganic carbon) are typically much higher than the rates of other comparable invertebrates as well as cnidarians (Fig. 1). This is not unexpected as the oxidation of both methane and sulfide have high oxygen requirements (stoichiometrically, methane oxidation typically requires two oxygens per methane molecule for complete oxidation and less to the degree that methane carbon is incorporated into organic carbon, while sulfide oxidation typically uses two oxygens per sulfide molecule). The ability of the animal hosts to support these high oxygen demands is a critical determinant of the rates of carbon fixation that can be achieved. For example, the oxygen uptake rate by *Riftia*, which is the highest among the chemoautotrophs in Fig. 1, is higher than routine rates for highly active animals such as loliginid squid and active fish (horse mackerel), even though *Riftia* is not using it for locomotion. For a sessile invertebrate, *Riftia* and other siboglinids have an astonishing and unique ability to take up and transport oxygen at very high rates.

Although these high oxygen consumption rates are not to support typical animal needs such as endothermy or muscular activity, they impose upon the host the same sorts of demands for oxygen uptake. The symbiont containing tissues are novel, high oxygen demand tissues within the context of these metazoans. In the remainder of this section we will further examine the functioning of chemoautotrophic and methanotrophic symbioses to evaluate which properties of these systems are associated with higher rates of carbon fixation.

Characteristics and functioning of siboglinid-chemoautotrophic endosymbioses

The highest rates of carbon fixation and oxygen consumption have been found in the hydrothermal vent clade of the siboglinids (Table 1). The members of this clade, represented by *Riftia*, *Tevnia* and *Ridgeia*, live in vent environments characterized by elevated temperatures and high fluxes of sulfide in the venting waters around the worms. Worms in this clade carry out gas exchange entirely across their plume, which is positioned at the turbulent interface between the venting water and the ambient deep-sea water (Childress and Fisher, 1992; Johnson et al., 1988). These worms typically have very large gill areas relative to their size (22 cm^2 g^{-1}). They all have very thin diffusion distances (ca. 2 µm) between the water and their hemolymph (Andersen et al., 2006; Andersen et al., 2002). These parameters support a high capacity for diffusion, being comparable to those of very active pelagic fishes, for example.

In contrast, the more basal hydrocarbon seep clade, Lamellibrachiidae, represented by *Lamellibrachia* in Table 1 (Black et al., 1997; McMullin et al., 2003; Rouse, 2001), live at lower temperatures with their basal ends buried deep in sediments and their plumes positioned well above the sediments. They have considerably lower rates of CO_2 and O_2 uptake and take up oxygen through their proportionally much smaller plumes and sulfide through their posterior extensions that have been dubbed “roots” (Freytag et al., 2001; Julian et al., 1999; Ortega et al., 2008). Their environment is stable and depletion of sulfide around the roots probably limits their autotrophic potential, though they do transport the endproduct of sulfur oxidation, sulfate, back into the sediments to further stimulate sulfide generation by sulfate reducing bacteria (Cordes et al., 2003; Dattagupta et al., 2008; Dattagupta et al., 2006). In the evolution of the vent siboglinids, gills apparently became enlarged and all metabolite exchanges were localized to the gill, enabling much higher metabolite uptake from the turbulent vent waters.

The physiological functioning of the vent siboglinids is portrayed in Fig. 2. The functioning of the seep worms would be similar with the uptake of sulfide and elimination of sulfate transferred to the posterior root structure. As adults, the siboglinids lack a gut or a mouth. They have a large circulatory system which pumps hemolymph through the gill and then to the trophosome where the bacteria are located in bacteriocytes. The trophosome is heavily vascularized with small blood vessels that come within a few µm at most of the individual bacteriocytes ensuring effective exchange of metabolites with the hemolymph (Arp and Childress, 1985). The trophosome accounts for between 10 and 30% of the wet tissue weight in *Riftia* depending upon the worm size, and the coelomic fluid is around 25% (Childress et al., 1984; Fisher et al., 1988a), while hemolymph is around 15% (J. J. Childress, unpublished). The coelomic fluid, which surrounds the trophosome, does not circulate but is in close contact with the hemolymph. The total hemoglobin concentration is much lower in the coelomic fluid, as it lacks the large 3.5 kDa hemoglobin that is found only in the hemolymph (Childress et al., 1991a). Also, both sulfide and nitrate concentrations are much lower in the coelomic fluid due to limited binding capacity resulting from lower hemoglobin concentrations. The coelomic fluid is thought to act as a metabolite reservoir to buffer short term fluctuations in uptake over the plume. For many inorganic ions, coelomic fluid and hemolymph are nearly in equilibrium though there are small, significant differences (Childress et al., 1991a).

In *Riftia*, oxygen diffuses through the gill surface and is bound to the very high oxygen affinity hemoglobins that transport it to the trophosome (Arp et al., 1990; Terwilliger et al., 1985). The very high affinity ensures loading of the hemoglobin at the gill surface when oxygen is available, but also limits the spontaneous oxidation of sulfide and restricts unloading when the plume is exposed to the anoxic venting waters. The vascular hemoglobins also bind sulfide with a very high affinity to sites different from those that bind oxygen (Arp and Childress, 1983; Arp and Childress, 1985; Childress et al., 1984; Fisher and Childress, 1984). This enables these worms to greatly concentrate sulfide in their blood (Childress et al., 1991a), and transport it to symbionts while preventing spontaneous reaction with oxygen (Fisher and Childress, 1984). This sulfide binding capacity serves to provide high concentrations of sulfide to the symbionts while protecting the symbionts from substrate inhibition, as was demonstrated in experiments showing much greater carbon fixation by isolated trophosome tissue in the presence of *Riftia* blood as compared to saline with equal sulfide concentrations (Fisher et al., 1989; Fisher et al., 1988b). These hemoglobins also have a high enough affinity for sulfide to protect cytochrome-c-oxidase from sulfide poisoning (Powell and Somero, 1983). The binding mechanism that was originally thought to involve binding to free sulfhydryl groups on the hemoglobins has now been shown to involve binding to zinc ions on the hemoglobins (Flores et al., 2005; Royer and Flores, 2007).

Both H_2S and HS^- are acquired by the host, but surprisingly the charged HS^- appears to preferentially diffuse through the gill tissue into the blood (Girguis and Childress, 2006; Goffredi et al., 1997b). The $\sum H_2S$ concentration in the vascular and coelomic fluids is limited by the binding capacity of their hemoglobins, which appear to bind HS^- (Childress et al., 1991a). Unlike the molluscan symbioses, *Riftia* does not accumulate thiosulfate in the presence of sulfide, indicating that the siboglinids are specialized to provide sulfide -the most reduced and hence energetic form of inorganic sulfur- to their symbionts , rather than reducing toxicity by oxidizing it to thiosulfate (Childress et al., 1991a). In sum, the hemoglobins provide a stable supply of sulfide, at high concentration and low chemical activity, to the symbionts that enables them to have much higher levels of carbon fixation than would be the case without the sulfide binding (Fisher et al., 1989).

Previous studies have also shown that when *Riftia* are exposed to adequate sulfide concentrations over time, the symbionts store a substantial fraction as elemental sulfur. This can reach concentrations greater than 10% of the wet weight of the trophosome (Fisher et al., 1988a) which is quickly oxidized if sulfide is withheld from the worms (Childress et al., 1991a). The primary end-products of chemoautotrophic metabolism are sulfate and hydrogen ions. These are moved out of the worms by active transport in the gill (Goffredi et al., 1999a).

As mentioned, the primary source of nitrogen for biosynthesis in vent siboglinids is typically nitrate (Girguis et al, 2000). Nitrate enters across the gill, apparently by diffusion, and is bound to the hemoglobin (Girguis et al., 2000; Hahlbeck et al., 2005). In the trophosome it is reduced to ammonia, which is found in high concentrations in the vascular and coelomic fluids (De Cian et al., 2000; Girguis et al., 2000; Lee and Childress, 1994) and is presumably used by the endosymbionts in synthesizing amino acids (Lee et al., 1999). When supplied with nitrate there is substantial leakage of ammonium ion and lesser leakage of nitrate into the surrounding water apparently diffusing down the gradient due to the higher internal concentrations (Girguis et al., 2000). This outward gradient explains why the siboglinids don't usually take up ammonium ion (Lee and Childress, 1994). There are also a variety of other nitrogenous compounds at high concentrations in the trophosome, but their function is not clear (De Cian et al., 2000; Girguis et al., 2000; Lee and Childress, 1994). Although nitrate is potentially an oxidant that the symbionts can use, experiments with intact symbioses in net autotrophic balance have failed to show an impact of nitrate on uptake of oxygen, sulfide or inorganic carbon (Girguis et al., 2000). Further, when the intact symbioses are kept under severely hypoxic conditions the symbionts are apparently unable to consume nitrate as shown by their failure to lower the hemolymph nitrate concentrations over time in the absence of external nitrate (J. J Childress, unpublished). Nitrate is also at much lower concentrations in hemolymph from worms with a surfeit of sulfide in their environment than is the capacity of these same worms to bind oxygen in their hemolymph and this alone would limit the role of nitrate as an oxidant (Hahlbeck et al., 2005). Thus nitrate seems to have at best a marginal role as oxidant in siboglinid symbioses but a critical role as the source of nitrogen for biosynthesis.

Inorganic carbon for chemoautotrophic carbon fixation is taken up as CO_2 across the gill, facilitated by carbonic anyhdrase (Goffredi et al., 1997a; Kochevar and Childress, 1996; Sanchez et al., 2007). In the hemolymph it is stored primarily as bicarbonate at the relatively alkaline pH of 7.4, concentrating $\sum CO_2$ by an "alkaline trap" mechanism (Childress et al., 1993b; Goffredi et al., 1997a). For example, at one site where $[\sum CO_2]$ in the water around the worms was 4.7 mmol l^{-1}, $[\sum CO_2]$ in the hemolymph was 30 mmol l^{-1}, and values up to 70 mmol l^{-1} have been found at other sites. This high concentration in the blood facilitates the transport of inorganic carbon to the symbionts in the hemolymph. The high bicarbonate concentrations in the hemolymph result in the animal apparently transporting Cl^- out to compensate for what would otherwise be substantial osmotic and charge imbalances (Goffredi et al., 1999a). Carbonic anhydrase also plays a role in the movement of inorganic carbon from the blood to the symbionts in the trophosome bacteriocytes (Goffredi et al., 1997a; Goffredi et al., 1999b; Kochevar and Childress, 1996; Sanchez et al., 2007). Via inhibitor experiments on live animals in pressurized aquaria and respirometer vessels, investigators showed the stoppage of CO_2 uptake when carbonic anhydrase was fully inhibited, confirming that carbonic anhydrases are essential for the movement of CO_2 into and through the worm's tissues at rates needed by the symbiosis (Goffredi et al., 1999b). In siboglinids, once the carbon has been fixed by the symbionts, it is probably not rapidly translocated to the host. Current models suggest that there is a complex but orderly symbiont life cycle taking place in the trophsome, and that organic carbon is transferred to the host through systematic digestion of the bacteria in the bacteriocytes (Bright et al., 2000).

One remaining essential aspect of this symbiosis is the elimination of hydrogen ions. Diffusion of CO_2 or H_2S into the hemolymph as well as the oxidation of sulfide will yield a substantial load of hydrogen ions. However, *Riftia* has very effective control of hemolymph pH, showing little deviation from pH 7.4 regardless of the sulfide or inorganic carbon concentrations under aerobic conditions (Goffredi et al., 1997a). Respirometer experiments using live *Riftia* demonstrated that hydrogen ion excretion in live animals is closely tied to sulfide oxidation and the rates of hydrogen ion excretion of this animal are unprecedented for a marine animal (Girguis et al., 2002). The use of transport inhibitors on live animals demonstrated the total elimination of proton elimination with the concurrent elimination of CO_2 uptake, and severe reductions in sulfide and oxygen uptake. High activities of proton ATPases have been demonstrated in the gill of *Riftia* (Goffredi and Childress, 2001). Even inhibition of K^+/H^+ ATPases in the less metabolically active seep worm *Lamellibrachia* rapidly stopped carbon fixation and sulfide uptake (P. R. Girguis, unpublished). In sum, substantial proton pumping capacity appears essential for thiotrophic endosymbioses, even for those with lower metabolic rates.

Whereas an abundant availability of sulfide and oxygen, as well as elevated temperatures, are major environmental properties at diffuse vents that enable high carbon fixation rates in vent siboglinids, the studies above show that physiological and biochemical adaptations of the animal hosts are required to take advantage of these properties to sustain high rates of carbon fixation. These include the hemoglobins that can bind oxygen and sulfide with high affinity to separate sites, controlling toxicity, providing the necessary high capacitances in the hemolymph, and providing sulfide to the symbionts to sustain symbiont metabolism. Moreover, morphological adaptive traits such as the large gill surface enable high diffusive fluxes of substrates and waste products. Finally a pronounced capacity to control hemolymph pH via high activities of proton ATPases is necessary for the survival of the host, as well as for concentrating inorganic carbon and keeping the hemolymph pH in a suitable range for oxygen and sulfide transport. It is likely that the seep worms have lower rates due to temperature, but more importantly the low fluxes of sulfide to their "roots" deep in the sediments. In these diffusion dominated systems, they simply do not have the same rate of substrate supply to support chemoautotrophic function that the hot vent species do. It is apparent that in the advection-dominated vent flows -with a continuous supply of sulfide and oxygen- the evolved functional modifications, aside from the elimination of roots, were ones primarily of degree, not of kind.

Characteristics and functioning of mollusc-chemoautotrophic endosymbioses

The molluscs have evolved chemoautotrophic endosymbioses in six different families, five bivalve ones and one gastropod family (Distel, 1998; Stewart et al., 2005). All of these endosymbioses contain the bacteria within bacteriocytes in the surface layer of gill cells. As presented in Table 1, all of these bivalve symbioses have much lower rates of carbon fixation and oxygen demand than do the vent siboglinids (we discuss the provannid gastropods below). The most obvious attribute shared by all of the molluscan thiotrophic and methanotrophic endosymbioses is that they have very large gills compared to non-symbiotic bivalves or gastropods. The available data on the gill size relative to the whole body are given in Table S1 for these endosymbioses, as well as a few non-symbiotic species. Endosymbiotic species have gills that range from 17 to 38% of their wet tissue weights, while the non symbiotic ones range from 5 to 15% with non-mytilids being at the lower end of this range. Within the family Mytilidae, the gills of the symbiont bearing species (subfamily Bathymodiolinae) are an almost 3 fold greater percentage of the total tissue weight than in the non symbiotic *Mytilus edulis*. Another criterion for comparing the gills is the gill surface areas relative to the body weights. Such a determination is available for only one endosymbiotic mollusk, *Solemya velum*, which exhibits an extraordinarily high surface area of 107 cm^2 g^{-1} (Scott, 2005). This compares with surface areas in the range of 5 to 15 $cm^2 g^{-1}$ in other bivalves (Booth and Mangum, 1978; Ghiretti, 1966) and 10 to 22 $cm^2 g^{-1}$ in *Riftia* (Andersen et al., 2002).

From this perspective it seems clear that the apparent capacity of molluscan lineages to evolve gills of immense size relative to the total mass and area of the animals without impairing physical functioning of the animal is a key component of the success of these molluscs as hosts for thiotrophic and methanotrophic symbionts. These gills are not, however, gills in the same sense as the plume gill of *Riftia* or the gills of fishes and cephalopods. In these other cases, the gill is the site of diffusion of gases into or out of the circulating vascular fluid, which transports these gases to and from the tissues where they are used or produced. In the case of these molluscan symbioses, little of the metabolite exchange goes through the gills to the hemolymph, as by far the majority is consumed by the symbionts within the surface layer of the gills. In this sense these molluscan gills are not, to a large extent, functioning as gills but rather as very large surfaces that are very well ventilated while being physically protected . This is further emphasized by the relatively large diffusion distances from water to blood observed in some of these symbioses. For example the vesicomyid *Calyptogena elongata* has a diffusion distance of about 6 µm (Childress et al., 1991b) while *Bathymodilus childressi* and *B. thermophilus* have diffusion distances of about 12 and 17 µm respectively (Fisher et al., 1987) which reduce their effectiveness in passing gases to and from the hemolymph. Thus, their critical importance is as a very expanded surface, which is continuously and effectively exposed to the highest concentrations of the needed metabolites that are available in their environments. This view of bivalve gills is consistent with the literature on the gills of mytilids, in which their gills are commonly regarded as being as being large primarily to facilitate filter feeding (Bayne et al., 1976). In fact, for *Modiolus demissus* it has been estimated that most gas exchange for the animal tissues takes place across the body surfaces and only 15% of the consumed oxygen is carried in the hemolymph, which lacks a respiratory protein (Booth and Mangum, 1978). Thus, in the molluscan endosymbioses considered here, the gill sizes and areas seem to be driven not by the need for gas exchange into the animal but by the need for a large, ventilated surface area to accommodate a substantial symbiont population.

The other property, which is almost universal in these symbiotic molluscan groups, is the presence of tissue hemoglobins in the gills (Dando et al., 1985; Hourdez and Weber, 2005; Wittenberg, 1985; Wittenberg and Stein, 1995). Only the mytilids lack gill hemoglobins. These hemoglobins have been reported to interact with both oxygen and sulfide in thysasirid, solemyid and lucinid bivalves (Dando et al., 1985; Doeller et al., 1988; Kraus and Wittenberg, 1990). It seems likely that the substantial concentrations in the gills of the five non-mytilid molluscan families with endosymbionts are important in facilitating the movement of oxygen and sulfide to the symbionts as well as controlling the activity of these substances in the environment of the symbionts.

We will now examine the major types of molluscan thiotrophic symbioses, emphasizing the key animal physiological characteristics which facilitate the symbiosis.

Vesicomyids

The Vesicomyidae is a very widely distributed and speciose bivalve family. Its members typically are found at the surface of reducing sediments which have reducing conditions near the surface. They have a very extensible foot, and use this to reach into the reducing areas of the sediment to access sulfide, which they take up across this foot and transport to the symbionts in the gills (Arp et al., 1984; Fisher, 1990). The hydrothermal vent vesicomyid clam *Calyptogena magnifica* extends its foot into cracks in the rocks or underneath mussels to access sulfide in weak vent flows. In turn they draw oxygen from the ambient water bathing their gills. Inorganic carbon is probably taken up across both the gills and the foot. Based on histological evidence and lysozyme activities, these clams apparently digest the symbionts (Fiala-Médioni et al., 1990; Fiala-Médioni et al., 1994). This overall scheme is in some ways similar to that of the cold seep siboglinids, and these clams are undoubtedly subject to the same limitations in accessing sulfide due to diffusion through the sediment. Indeed, sulfide uptake rates by two vesicomyid clams from cold seep environments are 2 and 11 µmol g^{-1} h^{-1}, comparable to the *Lamellibrachia* siboglinid seep tubeworm (Goffredi and Barry, 2002). Although there have been no measurements of net carbon dioxide or oxygen uptake in the presence of sulfide, their growth rates are not explosively fast like the vent siboglinids but are in a rather typical range for non-symbiotic shallow living bivalves (Lutz et al., 1988). The physiological functioning of this symbiosis is the most different of the six molluscan families (Fig. 3) (Childress and Fisher, 1992).

In addition to gill hemoglobins, vesicomyids have hemoglobin in cells in their vascular system to transport oxygen from the gills to the animal tissues. They also have a very large protein in the hemolymph that binds sulfide to a zinc moiety (Childress et al., 1993a; Zal et al., 2000). This protein concentrates sulfide into the hemolymph then releases it to the symbionts (Childress et al., 1993a). However, unlike the siboglinids, which do not produce thiosulfate to any extent, the vesicomyids do produce thiosulfate in the presence of sulfide and the symbionts metabolize it when deprived of sulfide (Childress et al., 1993a; Childress et al., 1991a).

Inorganic carbon uptake is facilitated by carbonic anhydrase in the gills (Kochevar and Childress, 1996). One way that the vesicomyids are very different from the siboglinids is that they regulate their hemolymph pH very poorly (Childress et al., 1993a; Childress et al., 1991a). While *Riftia* maintains a stable hemolymph pH with virtually any concentration of sulfide under aerobic conditions, the blood pH of vesicomyids quickly declines as sulfide increases in concentration. This relative lack of ability to deal with hydrogen ions would be expected to limit the potential rate of sulfur oxidation. In summary, the vesicomyids are functionally organized to draw sulfide from reducing sediments beneath the surface through their foot. They are adapted to situations where sulfide availability is generally low, and correspondingly appear to have limited rates of carbon fixation.

Other bivalves

The bathymodiolin mytilids are represented here by Fig. 4 with the other three families represented with additions to the figure as noted below. All of these species acquire sulfide via the gills, and all except the mytilids acquire their sulfide from the sediments in which they live. Thus they too have the general limitations associated with sulfide diffusion in sediments. All of these species oxidize sulfide to thiosulfate to control toxicity so the symbionts likely have access to both sulfide and thiosulfate. They all have carbonic anhydrase to facilitate CO_2 uptake (Kochevar and Childress, 1996). The mytilids do not burrow in sediments and so must draw their sulfide from the water around themselves. Only the mytilids are found at rocky hydrothermal vent sites, often from moderate or low flow areas but sometimes from higher flow areas with large supplies of sulfide. The mytilids are effective filter feeders (Page et al., 1991), while the other groups have quite reduced feeding and digestive abilities in most cases (Le Pennec et al., 1995). Only the mytilids lack gill hemoglobins. The mytilids, like the lucinids and thyasirids, probably rely entirely on digestion of the symbionts for transfer of fixed organic material while the solemyids rely on rapid leakage of material and distribution via the vascular system (Fisher and Childress, 1986; 1993). All of these symbioses seem to have relatively modest rates of carbon fixation, but the symbioses appear to be obligate in all cases and the reduced feeding and digestive systems of the lucinids, thyasirids and solemyids indicate that the symbioses fix enough carbon to reduce or eliminate the need for particulate feeding.

Provannid gastropods

The provannid gastropods *Ifremeria nautilei* and *Alviniconcha* species, are the only endosymbiotic gastropods. With the addition of gill hemoglobins to the schematic for sulfur-oxidizing bivalve symbioses other than vesicomyids, Fig. 4 also represents the state of our knowledge about them. These are very different species in terms of anatomy and ecology. *Ifremeria* has a relatively massive, heavily calcified shell, and is relatively inactive, living in cooler waters away from the active venting than *Alviniconcha* (Desbruyères et al., 1994; Podowski et al., 2009). In contrast *Alviniconcha* lives in vigorously venting water at higher temperatures, has an essentially uncalcified shell and is very active. Both have hemocyanin in their vascular system to transport oxygen to their tissues. *Alviniconcha* has much higher rates of carbon fixation and oxygen consumption, comparable to what we observe in siboglinids (Henry et al., 2008). These higher rates clearly echo the availability of substrates in its environment, which is one with considerably higher available concentrations and supplies of sulfide as well as higher temperatures (Podowski et al., 2009).

Characteristics and functioning of methanotrophic endosymbioses

Methanotrophic symbioses live in reducing environments as well, often close to symbioses with thiotrophic endosymbionts. A recent review summarizes the relatively small literature on these symbioses (Petersen and Dubilier, 2009). There have been reports of methanotrophs in one species of *Alviniconcha*, but these have not been confirmed in living organisms or tissues. When methane consumption was tested in live *Alviniconcha*, the result was negative (Henry et al., 2008). To date, there are two species of siboglinid, a very thin pogonophoran, which have been shown to have methanotrophic symbionts(Petersen and Dubilier, 2009; Schmaljohann and Flügel, 1987). In contrast there are a number of known methanotrophic symbioses among bathymodiolin mussels, including species with only methanotrophic symbionts (Childress et al., 1986), and species with both thiotrophic and methanotrophic symbionts (Distel et al., 1995; Duperron et al., 2005; Fisher et al., 1993). This is the symbiosis depicted in Fig. 5.

Methanotrophic symbioses must also oxidize sulfide (to thiosulfate), as it co-occurs in nearly every benthic environment that is rich in methane. The uptake of methane, however, is simple as there are no known binding proteins or uptake systems. It is reasonably soluble in water (ca. 2 mM at one atm pressure). The mussels, like many other mytilids, do not regulate either their oxygen or methane uptake very well so they require substantial concentrations of both to obtain a sufficient supply (Kochevar et al., 1992). As with other molluscan symbioses, the very large gills and high environmental methane concentrations in their habitats are key to hosting and sustaining methanotrophic symbionts.

Characteristics needed for endosymbiotic chemoautotrophy and methanotrophy and higher rates.

The primary requisite for sustaining high rates of chemoautotrophy is living in an environment with an abundant supply of sulfide and elevated temperatures in addition to oxygen. Both the hot vent siboglinids and *Alviniconcha* live in and are adapted to the most active warm water flows (as opposed to the vent “smokers”, where temperatures reach hundreds of degrees Celsius). The supply of sulfide in the venting waters far exceeds what they can capture, and the elevated temperatures promote much higher rates of host and bacterial metabolism. Oxygen and nitrate are available to them due to the turbulent, incomplete mixing of the vent and ambient waters in their microhabitats. The siboglinids have a classic higher metazoan layout in which gases are exchanged at a large surface area gill, concentrated in the hemolymph and transported by the circulation to the bacteria containing tissue where the gases diffuse to the bacteria. As discussed earlier, an essential key to this functioning is the hemoglobins, which bind sulfide and oxygen to separate sites with very high affinities. Without this there would not be sufficient capacitance for sulfide and oxygen in the blood to satisfy even very modest bacterial needs. A high capacity for controlling internal pH via excretion of hydrogen ions using proton ATPases is also essential to maintain the functioning of the hemoglobin, alkaline trapping of inorganic carbon and other aspects of the worms' functioning in the face of high production of hydrogen ions by the dissociation of carbonic acid and the oxidation of sulfide. In addition, the dominant species of hot vent siboglinids are relatively large animals, which probably assists them in bridging and accessing both the reducing and oxic waters at diffuse flows. In terms of rates of metabolite exchange, performance, and functioning one can perhaps consider them the "tuna fishes" of the chemoautotrophic world, with the supported performance being manifest as sustained, elevated carbon fixation rather than sustained, rapid swimming.

The functioning of *Alviniconcha* species is less well understood, but clearly they live in and are adapted to the highest flow areas in the warm vents where they are found. Their high activity levels and large sizes (up to at least 88 mm across), enables them to position themselves well within and possibly across the vent flow, and their uncalcified shells may allow much faster growth. They have very large gills with tissue hemoglobins that are likely involved in the uptake and movement of these metabolites to the bacteria. Preliminary data suggest that they have high rates of proton elimination as well (P. R. Girguis unpublished).

The five bivalve families and *Ifremeria nautilei* all appear to have lower rates of carbon fixation and oxygen consumption, though these rates are relatively higher than non chemoautotrophic invertebrates (as in Fig. 1) where they are the lower of the data points for chemoautotrophic symbioses. In general, all bivalves occupy environments with a relatively lower supply of sulfide and cooler temperatures even if they live around hydrothermal vents. The notable exception is the bathymodiolin mytilids, which as mentioned, are somewhat different in that they sometimes occupy higher flow areas at vents. Some bathymodiolin mytilids have methanotrophic symbioses, but still have relatively low rates of carbon uptake. This may be partially explained by the fact that the are much more capable filter feeders and less capable of maintaining oxygen uptake at low oxygen partial pressures. So in general they may be less well adapted to support chemosynthesis and more adapted for a mixotrophic existence.

As a group these bivalves and the provannids all have greatly increased gill sizes and contain the bacteria within specialized cells in the surface of these gills. These are not for the most part gills in the usual sense, i.e. organs for exchange of gases between water and blood. In these symbioses most of the gas exchange is undoubtedly limited to the bacteriocytes in the surface of the gills. From this perspective, the large gill areas are not organismal gas exchangers as such but rather very large surfaces, which are well ventilated and physically protected.

In summary it appears that the habitat and adaptations to the habitat are the first determinants of the rate of chemoautotrophic function. Hemoglobins, either circulating or tissue appear to be essential in the functioning of all but the bathymodiolin symbioses. All of these symbioses have much higher oxygen demands than do non-symbiotic species and this is an important factor in selecting for large gill surfaces and hemoglobins. For all of these symbioses below the euphotic zone, nitrate is readily available and readily utilized and for those in sedimented environments, ammonium is also available for the symbiont's needs. All of these symbioses except the methanotrophs also have carbonic anhydrase to facilitate CO_2 uptake. Proton ATPases are also important for eliminating protons but it appears that symbioses with lower rates may have less rigorous control of internal hydrogen ion concentrations.

Why no cnidarian sulfur or methane oxidizing symbioses?

Just as *Riftia* is the iconic chemoautotrophic symbiosis based on its domination of vent sites in the Eastern Pacific, corals and other anthozoans are the iconic photoautotrophic symbioses. Even though anthozoans can adapt to sulfidic environments and are found at the vents (Vervoort and Segonzac, 2006), no cnidarian chemoautotrophic endosymbioses have been found in spite of early and long-standing interest and effort. The extent and diversity of photoautotrophic symbioses among the cnidarians has led many investigators to ask why cnidarian-chemoautotrophic symbioses have not been found in any of the chemically reducing habitats studied to date. Here we present some considerations -from a physiologist's point of view- that may serve to explain this pattern.

As mentioned, the ability to tolerate sulfide in the environment is essential in chemically reducing environments. It would appear that toxicity is not a factor since cnidarians are found in and around vents and other chemically reducing environments.

It is also unlikely that inorganic carbon availability serves to explain the absence of cnidarian-chemoautotrophic associations. Inorganic carbon is readily available in seawater, and both photoautotrophic and chemoautotrophic endosymbioses use carbonic anhydrase to facilitate inorganic carbon assimilation. Moreover, given that photoautotrophic symbioses have reasonably high surface to volume ratios, it is unlikely that carbon acquisition will be limited.

However, the surface to volume ratios of cnidarians are unlikely to be sufficient to support high oxygen demands. As mentioned, cnidarians are characterized by the need to have a substantial surface occupied by the photoautotrophic symbionts and exposed to the light. These surfaces can be somewhat convoluted in corals, but shading precludes elaborate convolution and thus limits the possible surface area. Cnidarians lack active ventilation mechanisms, depending on environmental water movement or gross body movements to refresh the water near their surfaces. In fact, anthozoans do not regulate their oxygen consumption well at lower oxygen partial pressures (Shick, 1990). Over the range of oxygen partial pressures found in the interface habitats occupied by chemoautotrophic symbioses, they would be unlikely to be able to support the substantial oxygen demands of even modest levels of thiotrophic or methanotrophic metabolism. In brief, there is a lack of sufficient surface area, which would result in excessive diffusion distances from the water to the symbionts, and the lack of active ventilation and respiratory proteins preclude the possibility of storing and transporting either oxygen or sulfide. It is perhaps telling that the photoautotrophic symbioses with molluscs, namely the tridacnid clams and saccoglossan gastropods, have located the symbionts not in the gills but in the digestive tract emphasizing the very different physiological demands of these two kinds of autotrophic symbioses.

We therefore hypothesize that the physiological limitations of the cnidarian body plan as concerns oxygen uptake from the environment is a major reason for the absence of chemoautotrophic symbioses in anthozoans. These findings do not preclude the possibility that a heretofore undescribed cnidarian hosts chemoautotrophic symbionts. However, based on the observed physiological and biochemical demands of the symbionts on host metabolism, and the physiological and morphological attributes of known cnidarians, it is unlikely that a highly active population of chemoautotrophic endosymbionts could be supported by their host. In contrast, the evolution of the very large surface areas for gas exchange, which is often facilitated by respiratory oxygen and sulfide binding pigments, are a major physiological and anatomical property among chemoautotrophic symbioses which enables the high metabolic activity of these chemoautotrophic symbionts.

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Fig. 1. The oxygen consumption rates from Table 1 of the chemoautrophic endosymbioses (filled circles), standardized to 20°C using a Q_{10} of 2, plotted with oxygen consumption rates of a variety of non-symbiotic invertebrates and fishes measured at or standardized to 20°C as above. Each of the filled circles represents one species using data from Table 1. Reading from the bottom of the graph, the solid line represents data for medusae (Thuesen and Childress, 1994). The X symbols and short dashed line represent data from *Anthopleura elegantissima* (Towanda, 2008), *Metridium senile* (Sassaman and Mangum, 1972) *Ceriatheopsis americanus* (Sassaman and Mangum, 1974) and *Haliplanella luciae* (Zamer and Mangum, 1979). The medium dashed line represents the “invertebrate” data including annelids, bivalves, gastropods, crustaceans, and echinoderms used by (Gillooly, et al., 2001). The long dashed line represents data on routine metabolism of horse mackerel, *Trachurus trachurus* (Herrmann and Enders, 2000). The line at the top with very short dashes represents routine metabolism data for loliginid and ommastrephid cephalopods (Seibel, 2007). All consumption rates are expressed relative to the live weight of the animals.

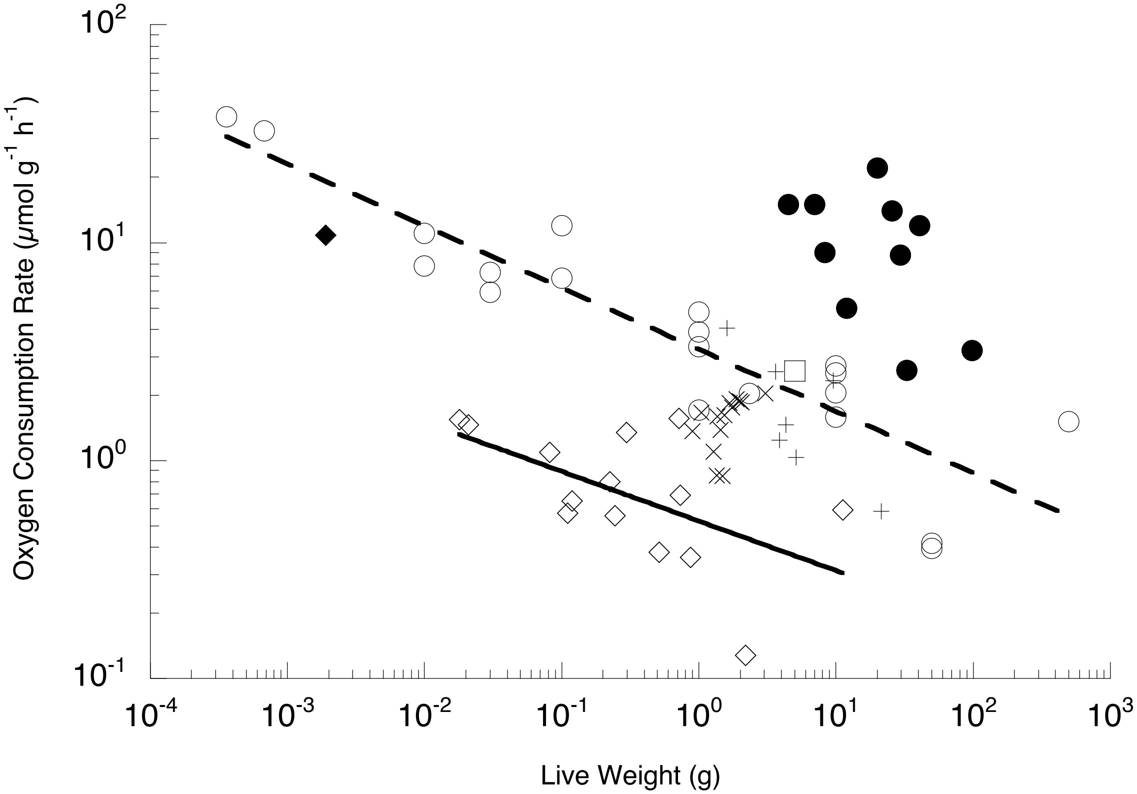
Fig. 2. A schematic of the physiological functioning of the hot vent siboglinid tubeworm, *Rifita pachyptila*. Light blue represents worm tissue with an intracellular pH of 7.4 (Goffredi et al., 1999a). Yellow represents the endosymbiotic bacteria (intracellular pH of trophosome bacteria and bacteriocytes is 7.0). Red represents the vascular fluid with a strongly defended pH of 7.4.. Pink represents the coelomic fluid with a pH near 7.4 but slightly above the hemolymph pH. The heavy arrows represent blood flow. The thin arrows represent either diffusion, chemical reactions, or chemical equilibration depending on the context. Thin arrows crossing tissue boundaries are diffusion. (The thin dark blue arrows represent minor fluxes of ammonium and nitrite ions.) A thin arrow with an open circle attached represents some sort of specific transport mechanism. Hb indicates hemoglobin and it is shown as binding and carrying sulfide, oxygen and nitrate. The word “digest” indicates that the symbionts are digested within the bacteriocytes in the gills. CHNO indicates organic matter from the digested bacteria. AA indicates amino acids. Nitrogen metabolism is separated from sulfide and carbon metabolism just for convenience in depiction. All other chemical labels have their usually accepted meanings. This schematic is representative of the functioning of the Eastern Pacific hot vent tubeworms, *Riftia pachyptila*, *Oasisia alvinae*, *Tevnia jerichonana*, and *Ridgeia piscesae*. As discussed in the text, the cold seep tubeworms have essentially the same physiological system except that the uptake of sulfide is through extensions of the posterior of the body deep into sulfide-rich sediments. Figure adapted from one in (Childress and Fisher, 1992).

Fig. 3. A schematic of the physiological functioning of the vesicomyid clam *Calyptogena magnifica*. Light blue represents animal tissue. Yellow represents the endosymbiotic bacteria. Red represents the vascular fluid. Pink represents the coelomic fluid. The heavy arrows represent blood flow. The thin arrows represent either diffusion, chemical reactions, or chemical equilibration depending on the context. Thin arrows crossing tissue boundaries are diffusion or unknown transport mechanisms. Hb represents hemoglobin and it is contained in erythrocytes depicted as ovals within the vascular fluid. BP indicates a separate protein, found in the vascular fluid which binds sulfide to a site

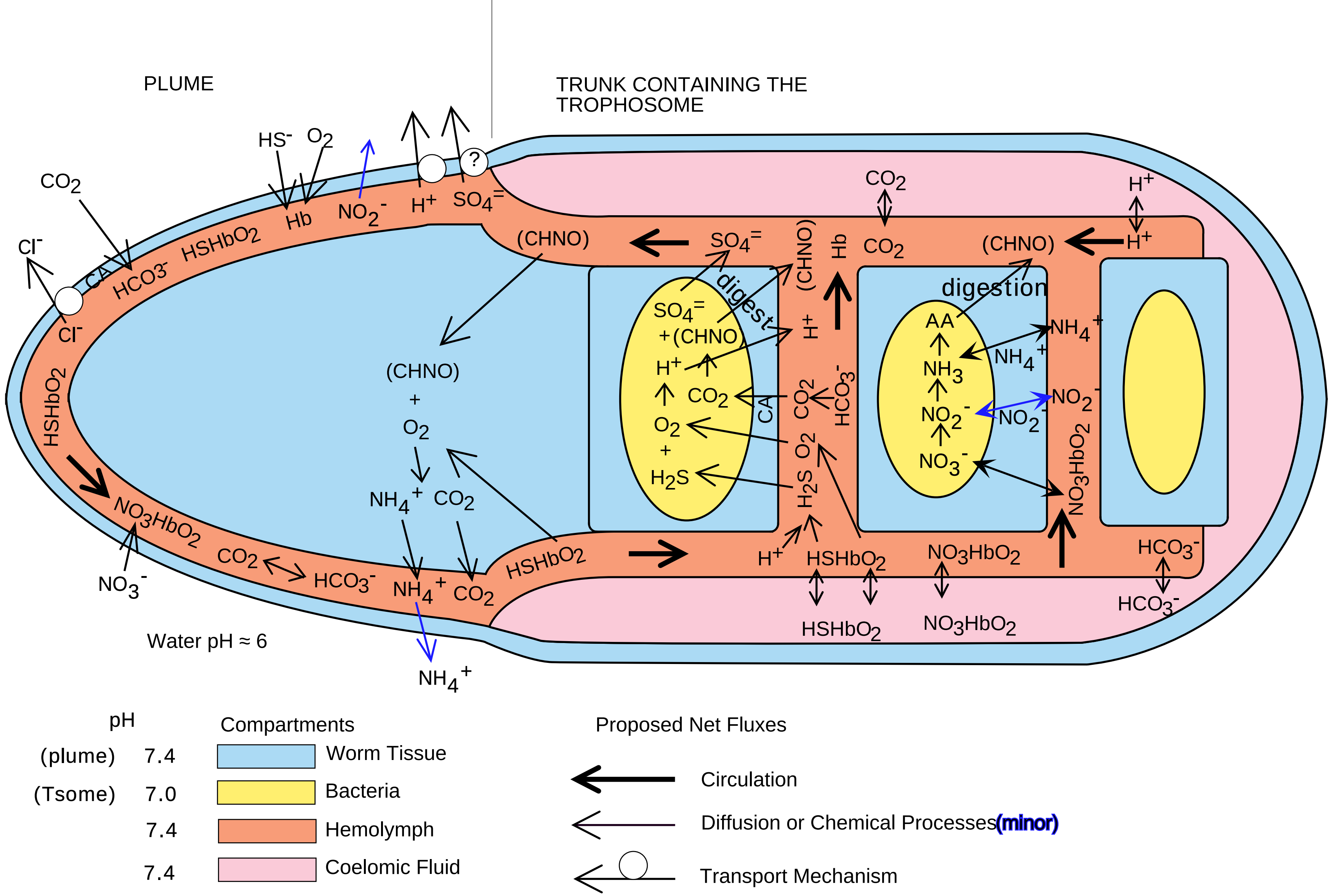
incorporating zinc. CA indicates carbonic anhydrase. SOX indicates the presence of a sulfide oxidizing activity which oxidizes sulfide to thiosulfate. The word “digestion” indicates that the symbionts are digested within the bacteriocytes in the gills. CHNO indicates organic matter from the digested bacteria The dominant uptake route for sulfide is believed to be across the foot which is extended into the substrate, while the other metabolites are taken up across the gills. This schematic is applicable to all members of the family Vesicomyidae.. Figure adapted from one in (Childress and Fisher, 1992).

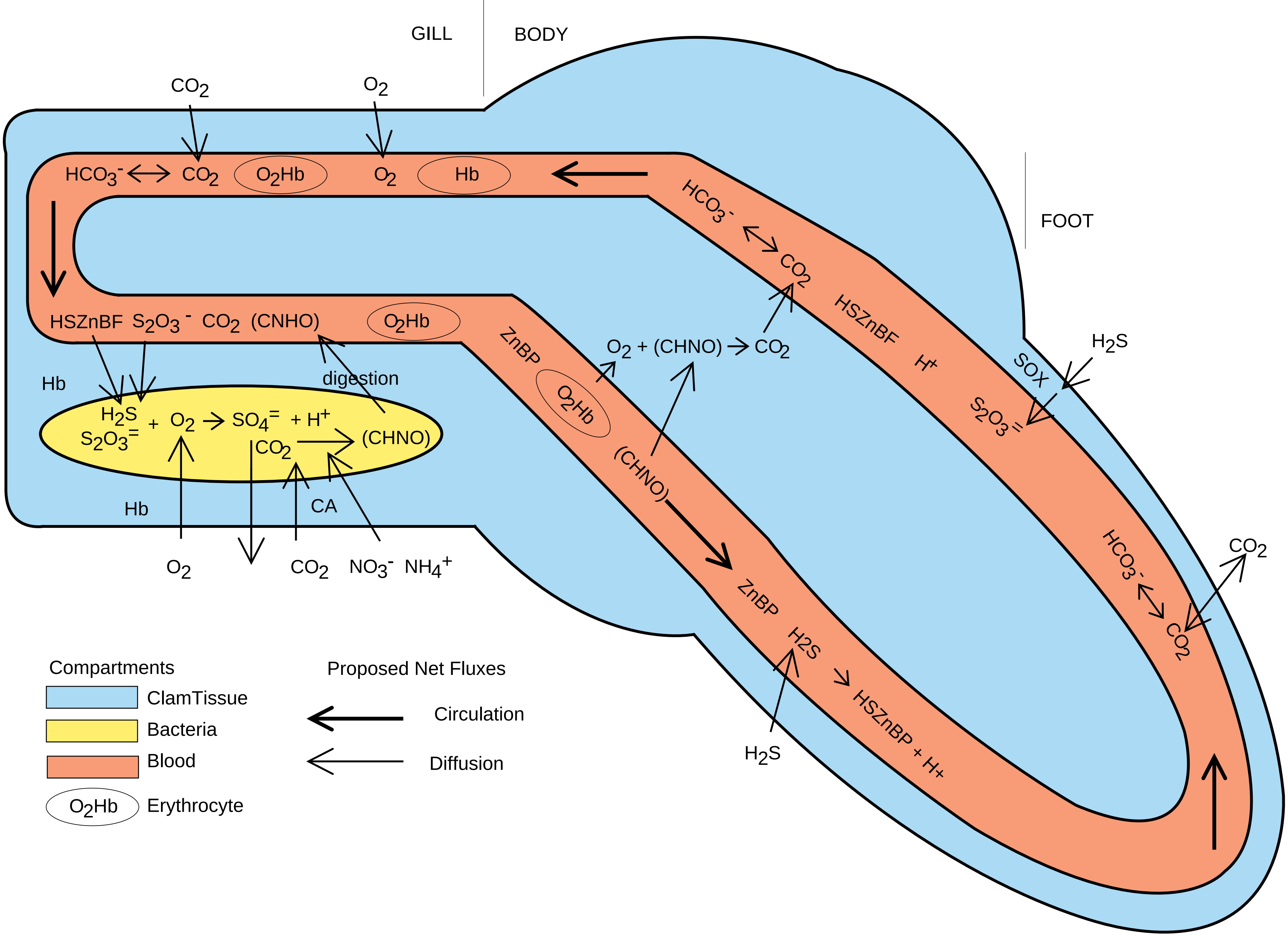
Fig. 4. A schematic of the physiological functioning of a mussel with sulfide oxidizing symbionts, such as *Bathymodilus thermophilus*. With certain modifications described below this is applicable to all of the molluscan endosymbioses except the Vesicomyidae. Light blue represents animal tissue. Yellow represents the endosymbiotic bacteria. Red represents the vascular fluid. Pink represents the coelomic fluid. The heavy arrows represent vascular fluid flow. The thin arrows represent either diffusion, chemical reactions, or chemical equilibration depending on the context. Thin arrows crossing tissue boundaries are diffusion or unknown transport mechanisms. CA indicates carbonic anhydrase. SOX indicates the presence of a sulfide oxidizing activity which oxidizes sulfide to thiosulfate. The word “digestion” indicates that the symbionts are digested within the bacteriocytes in the gills. CHNO indicates organic matter from the digested bacteria. All other molluscan endosymbioses, bivalves and gastropods, have gill hemoglobins (shown as (Hb)), often in high concentrations, which are very likely to be important in the uptake and sequestering of sulfide and oxygen in these symbioses. In addition both solemyid bivalves and provannid gastropods have substantial hemocyanin concentrations in their vascular fluids to supply the needs of the animal tissues for oxygen. Further, the solemyids, unlike all the other molluscan endosymbioses studied in detail, appear to rapidly transfer a large fraction of the chemosynthate via leakage from the bacteria instead of transferring it much more slowly after digestion of the bacteria as shown. Figure adapted from one in (Childress and Fisher, 1992).

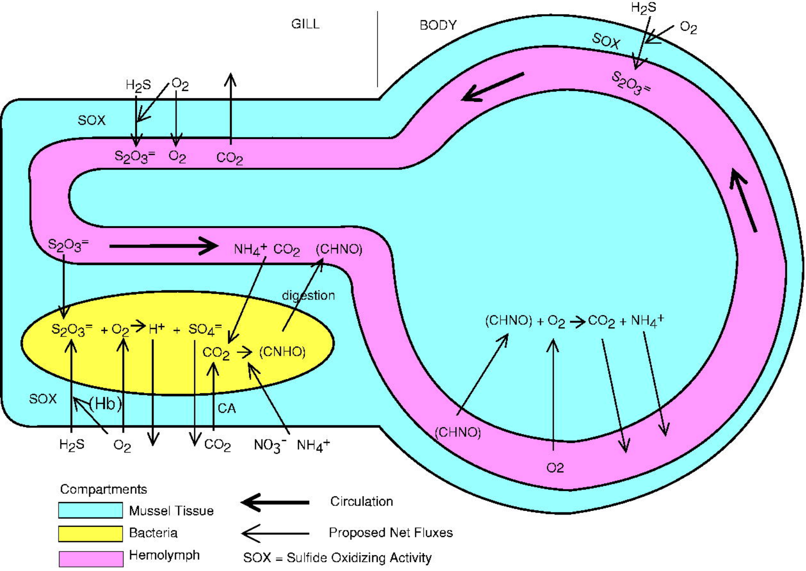
Fig. 5. A schematic of the physiological functioning of a mussel with methane oxidizing symbionts, such as *Bathymodilus childressi*. Light blue represents animal tissue. Yellow represents the endosymbiotic bacteria. Red represents the vascular fluid. Pink represents the coelomic fluid. The heavy arrows represent vascular fluid flow. The thin arrows represent either diffusion, chemical reactions, or chemical equilibration depending on the context. Thin arrows crossing tissue boundaries are diffusion or unknown transport mechanisms. SOX indicates the presence of a sulfide oxidizing activity which oxidizes sulfide to thiosulfate. The word “digestion” indicates that the symbionts are digested within the bacteriocytes in the gills. CHNO indicates organic matter from the digested bacteria. Due to substantial environmental sulfide concentrations, sulfide oxidation by the animal is required for control of toxicity of sulfide within symbiosis even though sulfide is not utilized by the symbionts.

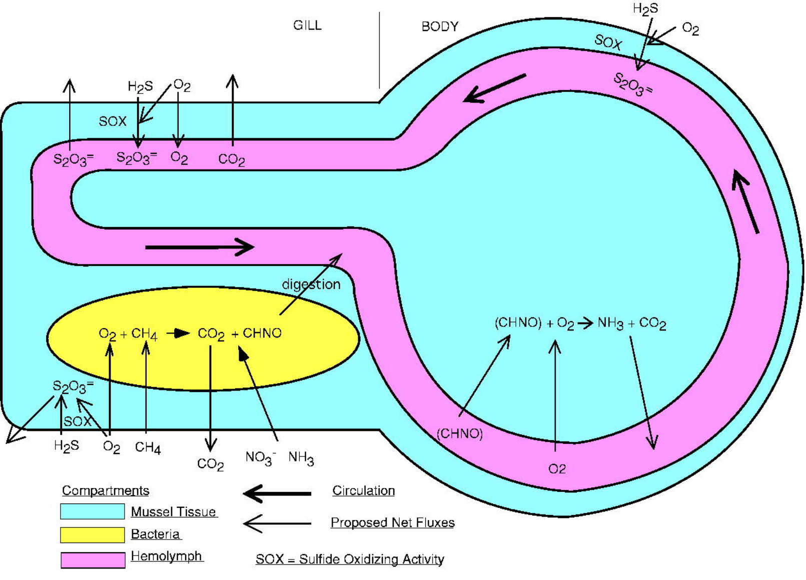


Oxygen Consumption Rate (μmol g⁻¹ h⁻¹)
Live Weight (g)
10^-1
10^0
10^1
10^2
10^-4
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3


PLUME
TRUNK CONTAINING THE TROPHOSOME
HS-
O2
CO2
Cl-
CA
HCO3-
HSHbO2
Hb
NO2-
H+
SO4=
?
(CHNO)
(CHNO)
+
O2
NH4+
CO2
HSHbO2
NO3HbO2
CO2
HCO3-
NH4+
CO2
NO3-
Water pH ≈ 6
NH4+
SO4=
digest
(CHNO)
Hb
CO2
CO2
(CHNO)
H+
H+
digestion
SO4=
+(CHNO)
H+
CO2
O2
+
H2S
CA
CO2
HCO3-
O2
H2S
H+
HSHbO2
AA
NH3
NO2-
NO3-
NH4+
NH4+
NO2-
NO2-
NO3HbO2
NO3HbO2
HSHbO2
NO3HbO2
HCO3-
HCO3-
pH
Compartments
Proposed Net Fluxes
(plume) 7.4
(Tsome) 7.0
7.4
7.4
Worm Tissue
Bacteria
Hemolymph
Coelomic Fluid
Circulation
Diffusion or Chemical Processes (minor)
Transport Mechanism


GILL
BODY
FOOT
CO2
O2
HCO3-
CO2
O2Hb
O2
Hb
HCO3-
CO2
HSZnBF
H+
HSZnBF S2O3 - CO2 (CNHO)
O2Hb
ZnBP
O2Hb
O2 + (CHNO) → CO2
SOX
H2S
S2O3=
Hb
H2S
S2O3=
+ O2 → SO4= + H+
CO2
(CHNO)
digestion
(CHNO)
Hb
CA
O2
CO2
NO3- NH4+
HCO3-
CO2
CO2
ZnBP
H2S
HSZnBP + H+
H2S
Compartments
ClamTissue
Bacteria
Blood
O2Hb
Erythrocyte
Proposed Net Fluxes
Circulation
Diffusion


GILL
BODY
H2S
O2
SOX
S2O3=
O2
CO2
H2S
O2
SOX
S2O3=
S2O3=
NH4+ CO2
(CHNO)
digestion
S2O3= + O2 → H+ + SO4=
CO2 → (CNHO)
SOX
(Hb)
CA
H2S
O2
CO2
NO3- NH4+
(CHNO) + O2 → CO2 + NH4+
(CHNO)
O2
Compartments
Mussel Tissue
Bacteria
Hemolymph
Circulation
Proposed Net Fluxes
SOX = Sulfide Oxidizing Activity



GILL
BODY
H2S
O2
SOX
S2O3=
O2
CO2
digestion
O2 + CH4 → CO2 + CHNO
(CHNO) + O2 → NH3 + CO2
(CHNO)
H2S
O2
CH4
CO2
NO3- NH3
O2
Compartments
Mussel Tissue
Bacteria
Hemolymph
Circulation
Proposed Net Fluxes
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Table 1. Representative sustained net rates (standardized to 20°C) of metabolite exhange for vestimentiferan and molluscan chemoautotrophic endsymbioses.

Species	Measurement Temperature °C	$\sum CO_2$ uptake (μmol g^{-1} h^{-1})	Relative C uptake (% Body C day^{-1})	O_2 uptake (μmol g^{-1} h^{-1})	H^+ equivalent elimination (μequiv. g^{-1} h^{-1})	Mass (g)
Riftia pachyptila	25	26.8	14	29	119	19-21
Tevnia jerichonana	25	18.6	8.3	21	(65)	6-8
Ridgeia piscesae	15	3.5	1.8	13.7	16.1	5-20
Lamellibrachia luymesi	7	2.4	1	5.3	(12)	3-6
Calyptogena magnifica	7	0.9	(0.3)			178
Bathymodiolus brevior	12	1.4	(0.5)	1.7		99
Solemya reidi	10	2.4	1	4.5		8
Bathymodiolus childressi	6	2.8 (CH_4)*	1.5	6		26
Alviniconcha hessleri	30	24.7	6.5	17.5	(41)	28-31
Ifremeria nautilei	13	0.7	0.3	1.4		25

Values in parentheses are less well documented values.
*C uptake based on a total CH_4 consumption of 4 µmol g^{-1} h^{-1} with 30% being released as CO_2.
Riftia values from (Girguis and Childress, 2006) as well as unpublished data.
Tevnia values are unpublished data of the authors.
Ridgeia values from (Nyholm et al., 2008).
Lamellibrachia values from (Freytag et al., 2001).
Calyptogena rates are based on ^{14}C fixation by gill pieces (Childress et al., 1991b).
Bathymodiolus brevior and *Ifremeria nautilei* rates from (Henry and Childress, 2008).
Solemya reidi rates from (Anderson et al., 1987).
Bathymodiolus childressi, which has methanotrophic symbionts, rates from (Kochevar, et al., 1992).
Alviniconcha (P. R. Girguis, unpublished).